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14. ABSTRACT

Multiple sclerosis (MS) is a devastating demyelinating disease in the CNS. We have recently discovered a new G-protein coupled receptor 17 (GPR17), whose activation was shown to inhibit myelination. In this project, we hypothesize that that GPR17 signaling activation results in blockade of remyelination. The specific aims of this study are: (1) to delineate the role of GPR17 in murine MS models of demyelinating diseases; and (2) to test the therapeutic potential for GPR17 agonists and antagonists in two MS models. During the first year of this project, we used cuprizone-induced demyelinating animal model to analyze the GPR17 function in remyelination. We evaluated the dynamics of GPR17 expression, and examined control and GPR17 null mice over the course of demyelination and remyelination process. Our study showed that deletion of GPR17 has a protective role during cuprizone-induced demyelination and enhances remyelination. Moreover, we found that GPR17 agonists inhibit OPC differentiation while GPR17 antagonists enhance oligodendrocyte differentiation in culture. These studies provide us a strong basis to pursue drug-based treatment of the demyelinating animal model during the next year of the project as outlined in the original proposal.

15. SUBJECT TERMS

remyelination, G-protein coupled receptor, GPR17, demyelinating animal model, GPR17 agonist and antagonist

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Introduction

Multiple sclerosis (MS) is thought to be an immune-mediated disease that is characterized by an immune attack on myelin sheaths in the central nervous system (CNS). There are several immune-focused treatment strategies for this disease, all showing partial benefit. It is becoming increasingly clear that we need treatment options that also enhance the process of CNS repair ("remyelination") and thus, it is critical to understand the underlying mechanisms that promote or deter such remyelination. The bHLH transcription factor Olig1 promotes oligodendrocyte maturation and is required for myelin repair [1, 2]. Recently, we have discovered a G-protein coupled receptor 17 (GPR17), whose function is to oppose the action of Olig1 and acts as a negative regulator for OPC differentiation [3]. We observed that sustained expression of GPR17 resulted in myelination defects in transgenic mice, whereas GPR17 knockout mice developed early onset of myelination. In addition, blocking of GPR17 was reported to enhance brain recovery after traumatic brain and spinal cord injury [4, 5]. Importantly, this molecule is increased in the context of inflammation, both in human MS and in its animal model, called EAE [3]. We showed that GPR17 is upregulated in MS plaques as compared to the white matter from non-neurological donor samples and normal appearing white matter from MS donors [3]. These observations suggest that GPR17 may serve as a potential therapeutic target for myelin repair in the CNS. At present, the signaling pathway mediated via GPR17 to block OPC differentiation is not fully understood. The proposed projects aimed at understanding biology of GPR17 signaling in animal models of MS with an emphasis on developing novel therapeutic approaches for promote remyelination in the CNS.

BODY

This is a two-year study with the following two specific aims:

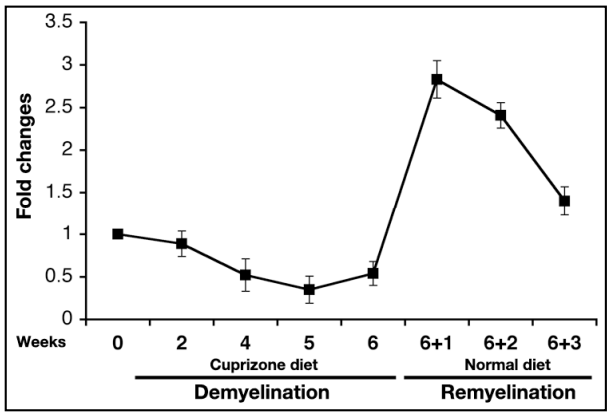
1. To delineate the role of GPR17 in murine models of demyelinating diseases.
2. To test the therapeutic potential for GPR17 agonists and antagonists in two models of MS.

As outlined in the statement of work (SOW), Dr. Lu's laboratory is responsible for experiments addressing the cuprizone-induced demyelination model of MS. Following are our research results from year 1 according to the tasks outlined in the SOW.

Task 1. Test the prediction that loss of GPR17 will diminish demyelinating pathology.

A) To delineate the role of GPR17 in murine models of demyelinating diseases, our group has carried out cuprizone-induced demyelinating/remyelinating study. Wildtype control mice and GPR17 null mice at age of 8 weeks were fed with cuprizone diet (0.2% cuprizone diet) for 1-6 weeks to cause demyelination and then switched to normal diet for 1, 2 and 3 weeks to promote remyelination. We first evaluated the dynamics of GPR17 expression during various stages of disease in control mice and found that GPR17 is substantially upregulated at the onset of remyelination as shown in Figure 1.

Figure 1. Correlation of GPR17 expression with progression of remyelination in the cuprizone-induced demyelinating model.
GPR17 expression detected by qRT-PCR using corpus callosum tissue from cuprizone treated wildtype animals (n=3) during 2, 4, 5 and 6 weeks of cuprizone treatment, and after animals were fed with normal diet for one (6+1), 2 (6+2) and 3 weeks (6+3).



B) To determine the role of GPR17 in remyelination, we have examined the wildtype control and GPR17 null mice over the course of cuprizone-induced demyelination and remyelination process. Our initial study with study of a cohort of mice (10 mice in each group) show that deletion of GPR17 leads to more resistance to demyelination in the animal treated with cuprizone, consistent with previous report that blocking GPR17 activity protected MCAo-induced brain injury [4, 5] (Figure 2). In addition, appearance of Mbp expression during the remyelination process appears to be accelerated in GPR17 knockout animals as compared to control (Figure 2). These results indicate that elimination of GPR17 enhances remyelination after cuprizone-induced demyelinating injury, and support our underlying hypothesis.

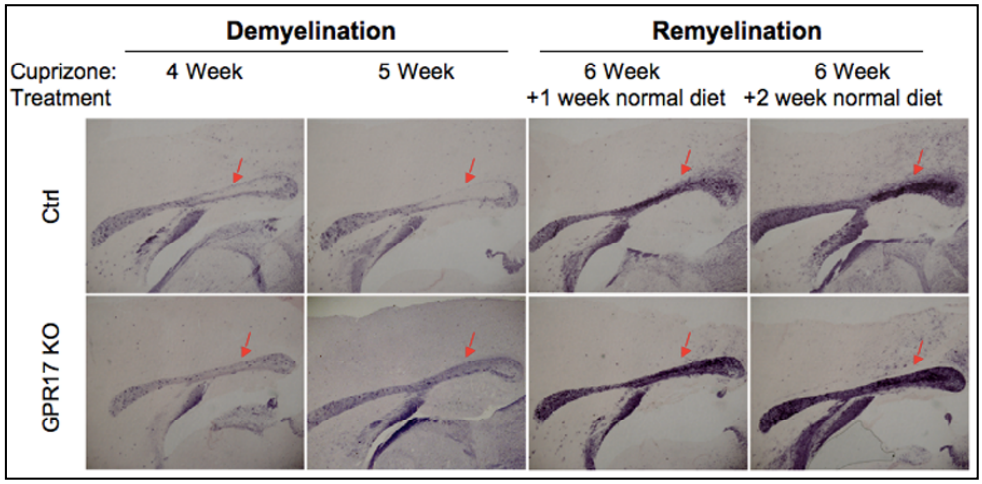


Figure 2: GPR17 knockout results in a protection against cuprizone-induced demyelinating injury and enhances remyelination
8 week old control (GPR17+/+) and GPR17 -/- knockout animals were treated with 0.2% cuprizone diet for demyelination as indicated. Animals were returned to the normal diet after 6 week treatment. The brains were harvested and processed for in situ hybridization to detect myelin marker myelin basic protein (*Mbp*) mRNA. In control mice, severe demyelination induced by cuprizone treatment as a sign of lack of *Mbp* expression in the caudal corpus callosum was observed, in contrast, *Mbp* expression in *GPR17-/-* mice is relatively maintained in the corpus callosum region, indicating a resistance to cuprizone-induced demyelinating injury in the absence of GPR17. Importantly, during the remyelination phase, more robust *Mbp* expression in the corpus callosum was detected in GPR17KO animals as compared to the control.

Task 2. To test the therapeutic potential for GPR17 agonists and antagonists

2A) As stated in the Task 2a (In vitro modeling of GPR17 blockade) in SOW, we obtained GPR17 agonists and antagonists and began to treat oligodendrocyte precursor culture with them. Treatment of GPR17 agonists (e.g. leukotriene $LTD_{4)}$ was found to block OPC differentiation in culture (Figure 3), while treatment of GPR17 antagonists (e.g. Montelukast) enhanced OPC differentiation.

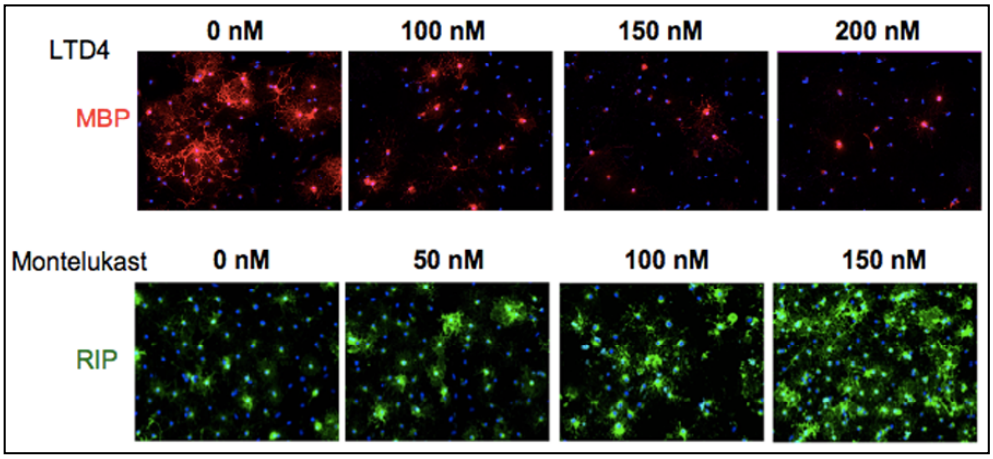


Figure 3: Effects of GPR17 agonists and antagonists on oligodendrocyte differentiation in vitro
(Upper panel) Rat OPCs isolated from neonatal pups were cultured in a differentiation condition (Sato medium supplemented with 15 nM thyroid hormone T3 and 10 ng/ml ciliary neurotrophic factor) and treated with a GPR17 agonist LTD4 at indicated concentrations for 5 days. Cells were stained with anti-MBP antibody. (Lower panel) Rat OPCs were cultured in the OPC growth medium in presence of 10 ng/ml PDGF-AA and treated with a GPR17 antagonist Montelukast at indicated concentrations for four days. Cells were then stained for differentiated oligodendrocyte marker RIP.

2B) Task 2b: Testing GPR17 agonists and antagonists in vivo. We are treating the GPR17 agonists (leukotrienes LTD_4) and antagonists (Montelukast) in the context of cuprizone induced demyelination animals, and beginning to examine the effects of GPR17 agonists and antagonists on remyelination process as planned. To avoid the potential toxicity of drugs, we currently are optimizing the drug dosage for the study. In addition, we are also using in vitro myelination co-culture system to evaluate the effects of GPR17 agonists and antagonists on oligodendrocyte myelination using in vitro co-culture system.

Key Research Accomplishments

- GPR17 expression is upregulated at the onset of remyelination during the course of demyelination/remyelination in the cuprizone-induced demyelinating animal model of MS.

- Knockout of GPR17 in mice has a protective function during cuprizone-induced demyelination injury and accelerates the remyelination process.

- GPR17 agonists inhibit OPC differentiation while GPR17 antagonists enhance oligodendrocyte differentiation in culture.

Reportable Outcomes

None thus far. We anticipate multiple submissions [scientific reports and grants] during the next year of the project.

Conclusion

During this funding period, we have made progress to delineate the role of GPR17 in the context of demyelination/remyelination in a cuprizone-induced animal model of MS. We have determined the dynamics of GPR17 expression over the course of de/remyelination. We observed that GPR17 is upregulated at the onset of remyelination. Moreover, we showed that GPR17 knockout in mice has a protective function during cuprizone-induced demyelinating injury compared to the control mice, and enhances remyelination. In addition, in vitro blocking GPR17 activity by GPR17 antagonizing drug enhances OPC differentiation and maturation. These data encourage us to pursue our study with the initial goal of developing a novel and effective strategy for myelin repair.

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